A DIRECT RELATIONSHIP BETWEEN PANTOTHENATE CONCENTRATION AND THE TIME REQUIRED TO INDUCE THE PRODUCTION OF PANTOTHENATE-SYNTHESIZING "MUTANTS" IN YEAST[1]

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This paper describes experiments indicating that the concentration of pantothenate bears a direct relation to the time required to restore pantothenate synthesis in a yeast cell. Various members of the pedigree shown in Table II[2] were grown in batches of Burkholder's medium made up with the following amounts of pantothenate added per liter: 100, 50, 20, 10, 5, 2, 1, 0.5, and 0 γ. Each tube was inoculated in a uniform manner with a loop. Three hundred colonies grew from each loopful of cells on plating, but since the haploid cells were typically aggregated the total number of cells was probably less than 1500.

Figure 3 shows the results with S. *cerevisiae* (culture No. 1), the turbidity being plotted against time in hours. The graphs are made by plotting the average of the turbidity produced in duplicate tubes, except in a few cases in which the tubes were so widely different that averaging did not seem to be a permissible practice. Usually the readings differed by only a few units and averaging was obviously acceptable. After 45 hours, growth is practically completed in the media containing 50 and 100 γ of pantothenate, but it is fully 75 hours before appreciable growth is recorded in the tube without the added pantothenate. This culture had previously been characterized as a synthesizer of pantothenate. These data show that diagnosis depends largely on the time at which readings are taken. Comparison of the 100 γ and 0 γ tubes at the end of 45 hours would have resulted in characterizing this particular organism as a "nonsynthesizer" of pantothenate. The relationship between the amount of added pantothenate and the time at which growth begins is quite clear, since the curves are all closely parallel during early and logarithmic growth and overlapping occurs only after the logarithmic phase of growth has been completed. There is a sharp difference between the time at which growth begins in the tubes containing 0.5 and 1 γ of pantothenate per liter as well as between growth in tubes containing 1 and 2 γ of pantothenate per liter.

The culture of S. *cerevisiae*, whose reactions are recorded in fig. 3, was induced to sporulate, and similar tests with the four haplophase cultures are shown in figs. 4 and 5. Cultures No. 3 and No. 4 are remarkably similar in behavior. According to previous techniques, these would have been classified as "nonsynthesizers" because growth in the absence of pantothenate did not begin until after 250 hours. The particularly interesting feature of the behavior of these cultures is the direct relation between the length of the delay before growth begins and concentration of pantothenate in the medium.

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[2]See preceding paper.

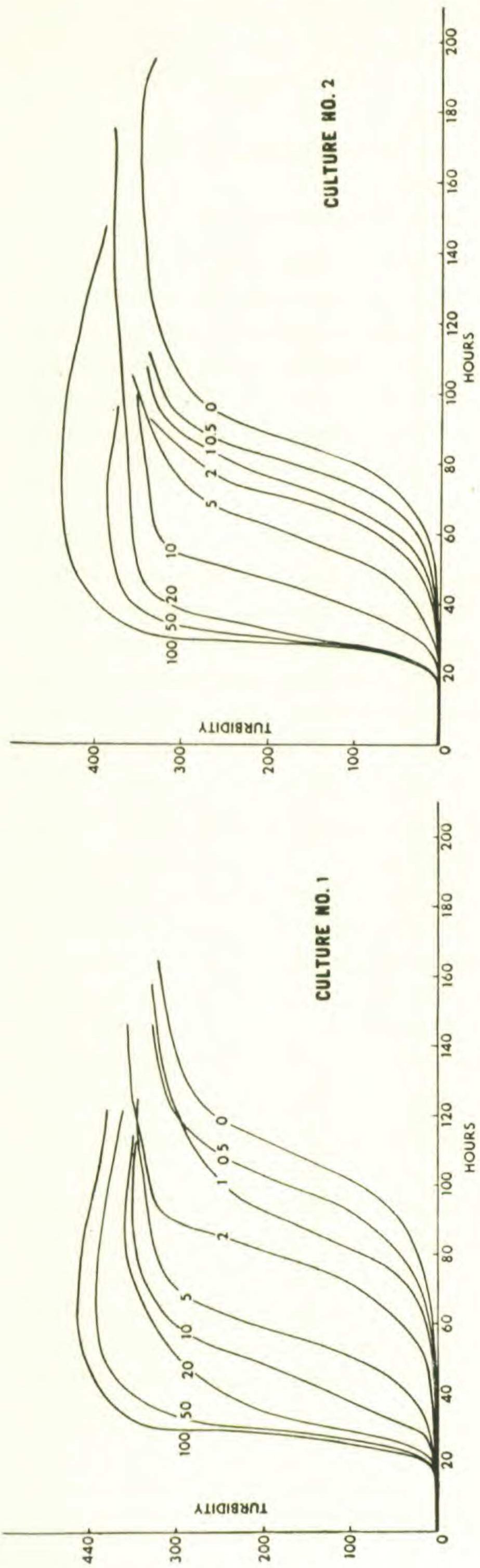

Fig. 3. The growth of cultures Nos. 1, 2, 7, and 10 in Burkholder's medium containing different concentrations of pantothenate. The solid lines represent the original inoculations; the dotted lines represent transfers from the 100 to 0.

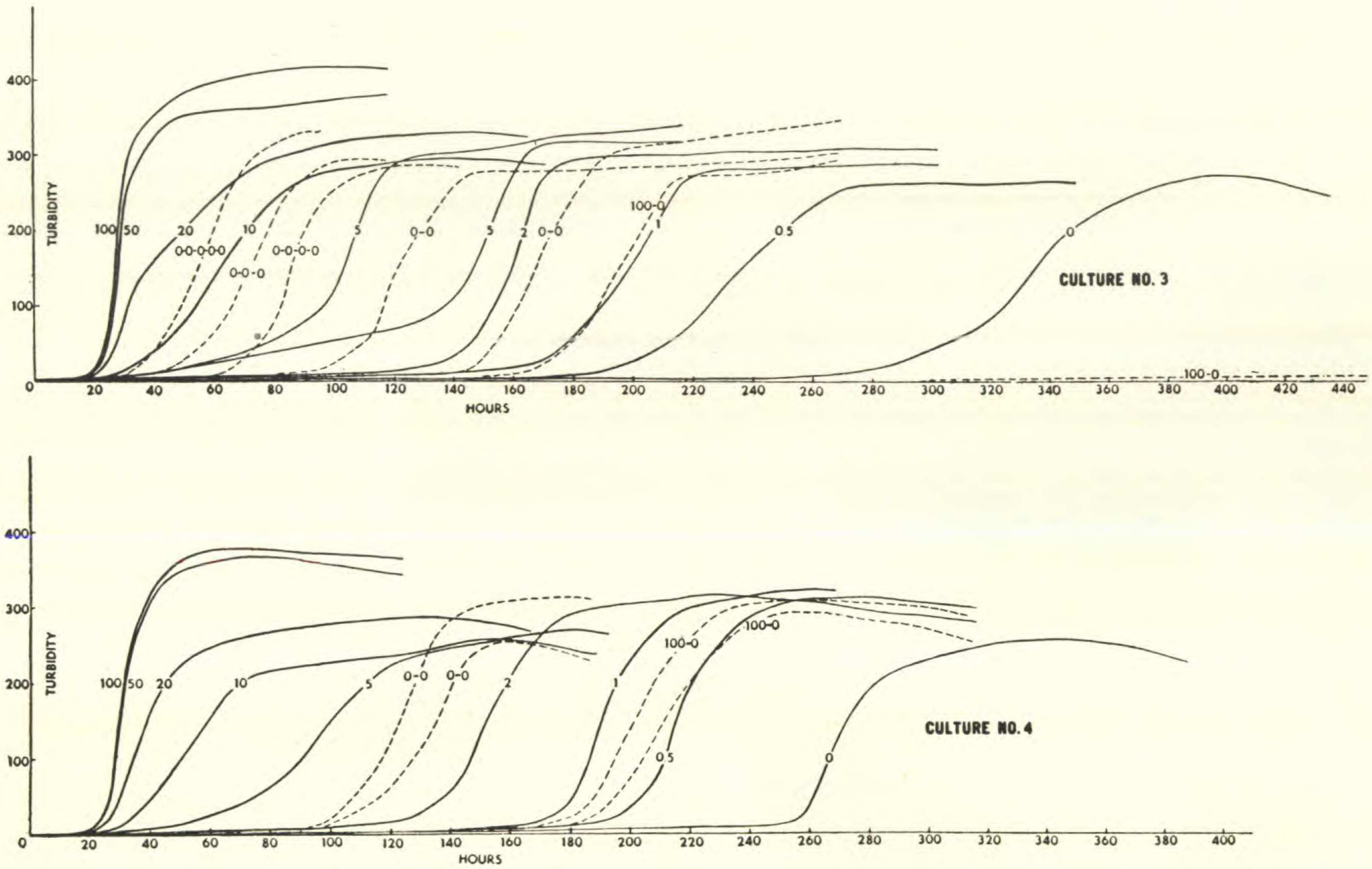

Fig. 4. Growth of cultures Nos. 3 and 4 on Burkholder's medium containing different concentrations of pantothenate. (The solid lines represent the original inoculations; the dotted lines represent transfers from these inoculations as indicated, 100–0 being a transfer from 100 to 0).

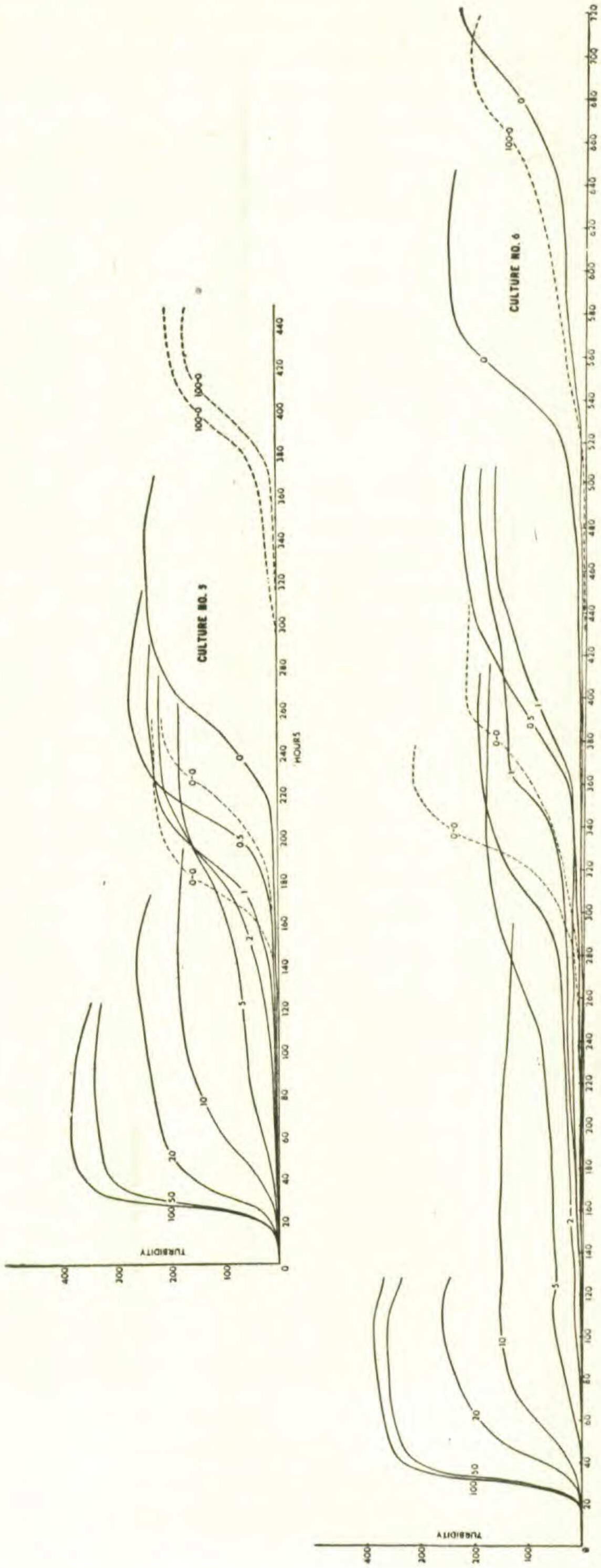

Fig. 5. Growth of cultures Nos. 5 and 6 on Burkholder's medium containing different concentrations of pantothenate.

In culture No. 5 (fig. 5) the different concentrations of pantothenate also bear a direct relation to the delay before growth begins. A similar picture exists for culture No. 6 (fig. 5), except that in the concentration of 1 γ and 0 γ per liter the duplicate tubes differed so markedly from each other that it was not permissible to average the results. This is one of the few cases in which growth in 1γ per liter in one of the duplicate tubes occurred later than that in the tube containing 0.5 γ per liter.

The behavior in culture No. 5 (fig. 5) shows an almost ideal example of general tendency of the "delayed" cultures. Growth in media containing 100 and 50 γ per liter takes place at a very rapid rate. In the medium containing 20 γ the rate is somewhat decreased, and in 10 γ considerably so. A further decrease occurs in 5 γ per liter, so that there is a continual decrease in rate of growth in the 50, 20, 10 and 5 γ media, respectively. The case is quite different in the 2, 1, 0.5 and 0 γ media, where beginning of growth is delayed more and more as the concentration decreases but once growth begins the rate is uniform and more rapid than in the 5 γ medium. The decreasing rate in the first five curves indicates that where there is an excess of pantothenate the growth bears a direct relation to the concentration of pantothenate, indicating that synthesis is suppressed when more than 5 γ per liter are present. (See also culture No. 4, fig. 4).

The rate of growth in the last four curves is practically identical, but the delay before growth begins bears a direct relation to the concentration of pantothenate. This is interpreted to mean that in each of the last four curves the growth begins after the induction of a "mutation" which possesses the ability to synthesize pantothenate and that the rate of growth depends on the synthesis of the vitamin by the cell. The basic assumption for this interpretation is the view that *de novo* mutations from inability to ability to synthesize are extremely infrequent and the mutations observed in the laboratory are practically all "loss" mutations. On this assumption, an agent which produces regular and precise changes in cells from "nonsynthesizers" to "synthesizers" does not produce a change of a completely non-existent locus to a synthesizing locus but merely acts to bring a partially degraded or temporarily inactive gene into functional activity. The rate of growth is independent of the concentration of pantothenate originally present in the medium (below 2 γ per liter). However, the time at which the "mutation" is induced (the "delay") depends on the concentration of pantothenate present; possibly directly on the number of molecules of pantothenate impinging on the gene. In a medium containing 1 γ per liter, more molecules would collide with any given surface than in one containing 0.5 γ per liter. The fact that only a small number of cells was used in inoculating the tubes and that easily detectable and constant differences exist between the low concentrations indicate that mutation occurs in many of the viable cells in a given tube at approximately the same time, rather than that one cell mutates and then outgrows its neighbors. If the latter were the case, the curves would overlap and the precise differences between the different concentrations would not be detectable. It

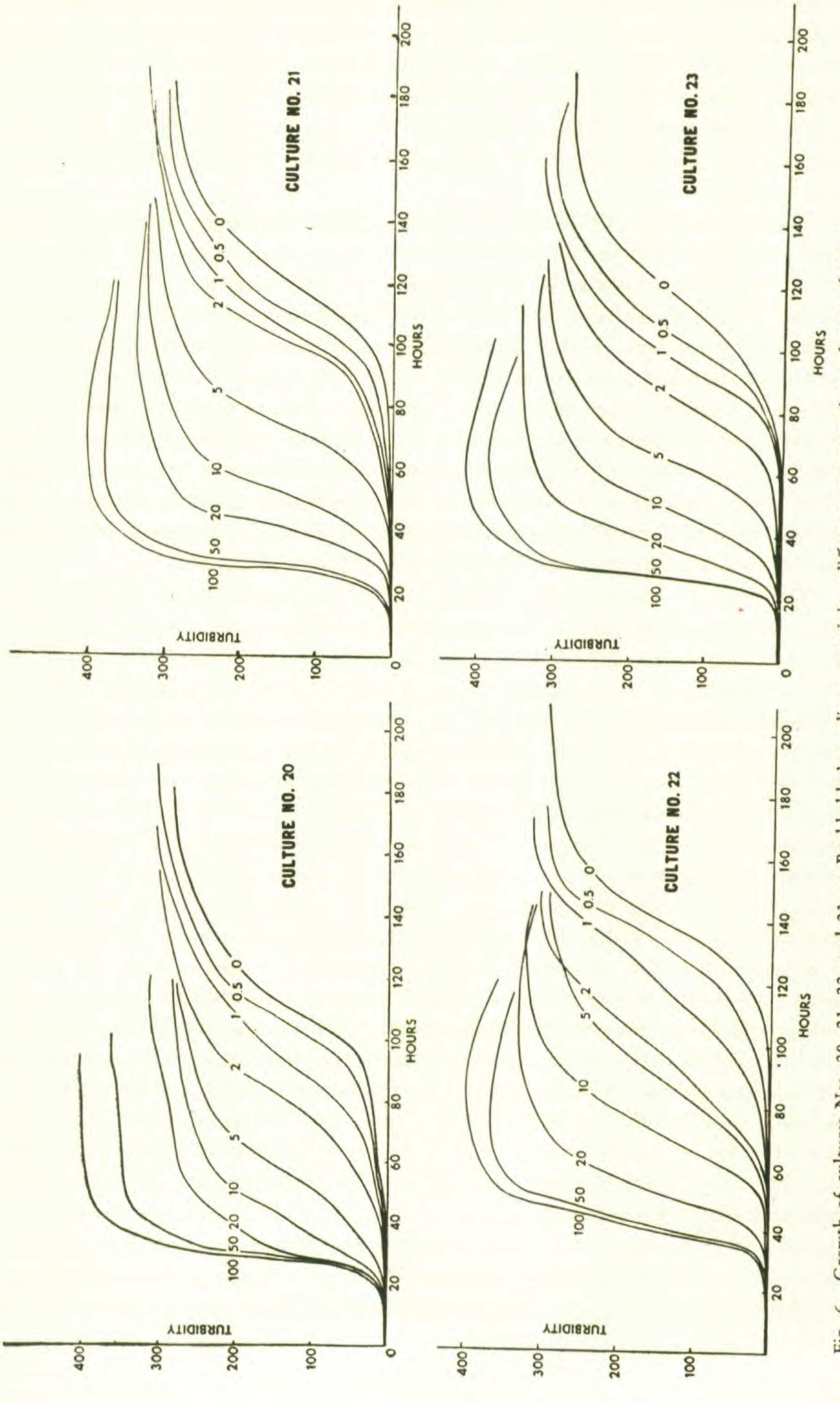

Fig. 6. Growth of cultures Nos. 20, 21, 22, and 23 on Burkholder's medium containing different concentrations of pantothenate.

is, of course, difficult to call these organisms "mutants" because the specific test for mutation is segregation. When transfers from the culture tubes without the added pantothenate were made to similar tubes likewise without added pantothenate (0–0) growth occurred about 150 hours sooner in the second than in the original test. These curves of growth are shown as dotted lines on the graph. As the yeast was transferred serially in the 0 medium, the delay before growth started was further shortened. The fifth transfer (0–0–0–0–0) began to grow sooner in the medium without the addition of pantothenate than the cultures which had been originally classified as synthesizers.

The original cultures came from a slant of yeast extract agar. Cells were suspended in 10 cc. sterile distilled water, and transfers to Burkholder's medium were made with a loop of this diluted suspension to insure against transfer of vitamins. A small but uniform number of cells was transferred in each loop. Irrespective of the concentration of pantothenate in Burkholder's synthetic media none of these original transfers failed to grow, but each grew after the delay indicated on the graphs. Many other transfers were subsequently made from one tube of Burkholder's synthetic medium to another with the same concentration of pantothenate; all these resulted in growth. Generally speaking, 0–0 transfers began growth much sooner than the original transfer, indicating that a "mutation" had occurred in the first transfer and that growth began due to the "mutation" or that some new channel of synthesis was established which became more efficient with continued use.

Saccharomyces carlsbergensis (culture No. 2, fig. 3) is an undelayed synthesizer of pantothenate, and growth in all concentrations of pantothenate is completed before 100 hours. The single haploid offspring of *S. carlsbergensis* (No. 7, fig. 3) is smiliarly an undelayed synthesizer but is spectacularly capable of utilizing any available pantothenate, as is shown by the beautifully parallel curves on the different concentrations. A hybrid between undelayed synthesizer (culture No. 7) and delayed synthesizer (No. 5) produced the hybrid culture No. 10, which was an undelayed synthesizer (fig. 3). When four haploid progeny from hybrid No. 10, cultures Nos. 20, 21, 22, and 23 (fig. 6), were tested, all showed the ability to use whatever pantothenate was available, as evidenced by the parallel nature of the curves for different concentrations. However, these four progeny were all undelayed synthesizers of the vitamin, and no clear-cut Mendelian segregation occurred. This does not necessarily mean that the difference is not one under gene control, for this pedigree is one in which gene transformation frequently occurs. This matter is being dealt with in an article now in press (Lindegren and Lindegren, '47). The pantothenate character segregates regularly in other pedigrees in which Mendelian segregation of other gene-determined characters normally occurs.

DISCUSSION

Non-Random "Mutation."—Mutations are generally supposed to result from random changes in the gene which occur independently of substrate with a specific

frequency. The probability that spontaneous or induced mutations would produce adaptive or "progressive" changes in a gene are generally thought to be about as likely as that the act of throwing a wrench at a motor would result in an improvement in the machine. Skoog and Lindegren ('47) have presented evidence indicating that mutation to glucose utilization was influenced by the nature of the substrate. The above data suggest that "mutation" which enables the cell to synthesize pantothenate depends directly on the concentration of pantothenate in the environment. The "mutations" induced by pantothenate are quite different from the ordinary recessive mutations used in genetical Mendelian analysis; they may merely be the result of the addition to the gene of one of its essential components. Such a component might correspond to what I have called the *cytogene*.

This presupposes that pantothenate synthesis is under genetic control. Most previously described "vitaminless" mutants are probably genotypes unable to survive in the deficient synthetic medium. The genotypes which we described here synthesize pantothenate when the level of pantothenate in the medium drops below a certain minimum. However, it is suggested by the data that some pantothenate (either in the cell or in the medium) must be present before the synthesizing mechanism can operate.

Organized versus Molecular Genes.—The gene is probably a loosely organized complex structure rather than a precisely definable chemical compound. The tendency to conclude from (1) the experiments of Stanley (in which an isolated crystalline nucleoprotein was shown to produce the same effect as tobacco mosaic virus) and from (2) the experiments of Avery, McLeod, and McCarty (in which a nucleic acid was shown to be capable of transforming one type of pneumococcus into another) that the gene is either a crystalline nucleoprotein or a nucleic acid disregards the possibility that the nucleoprotein and the nucleic acid may be only a part of the organized structure making up the gene. The fact that thousands of molecules of the mosaic virus nucleoprotein are required to produce a single infection has been interpreted to result from the difficulty of securing infection with a single particle. An alternative interpretation is possible: it may be that only one particle in a thousand of the "purified" preparation is so organized that it is capable of infection. In the pneumococcus experiment the transformation may have been achieved because the complex which comprised the gene producing the smooth mutant form was brought into functional activity by the addition of a single nucleic acid, just as a machine can be made to operate by adding a single nut. This does not mean that the nucleic acid is the gene, any more than the nut is the machine. Our experiments with pantothenate show that by adding molecules of it to a suspension of yeast cells a cell incapable of synthesizing pantothenate could be transformed into one capable of performing the synthesis. The fact that a gene-controlling synthesis has become functional may not mean that a gene has been added but merely that one component of the complex which makes up the gene has been supplied. This component, though essential, may be only a part of the total organized structure.

CONCLUSIONS

The evidence presented above indicates that in the presence of a large excess of pantothenate no synthesis of pantothenate occurs although growth of cells by utilization of the available pantothenate goes on at a very rapid rate. At concentrations not in excess of, but greater than the minimum required for growth, the cells do not synthesize, and the rate at which they grow is determined by the amount of pantothenate supplied. When the concentration of pantothenate is less than the minimum required for growth the cells "mutate" so that they are able to synthesize pantothenate and grow. The time required for this "mutation" to take place is determined by the small amounts of pantothenate which are present in the media. The data may not completely exclude the possibility that only a small fraction of the population has been affected and that selection has been an important factor in the phenomenon; further tests of this view are in progress. The present indications support the view that a large fraction of the population is involved and if this be true, pantothenate can be regarded as an agent which acts to repair a partially degraded gene. The data suggest that in the complete absence of pantothenate neither synthesis nor growth can begin. (The cells in the medium to which no pantothenate has been added did not necessarily begin growth in the absence of pantothenate, for each cell probably carried a sufficient amount to initiate growth.) Synthesis occurs in Burkholder's medium only if enough pantothenate to initiate synthesis but not enough to suppress it is present.

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